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MAMMALIENS (ZTGFSS9)

(54) MAMMALIAN TRANSFORMING GROWTH FACTOR BETA - 9

(ZTGFSS9)

(57) Novel mammalian Ztgfβ-9 polypeptides, polynucleotides encoding the polypeptides, and related compositions and methods including antibodies and anti-idiotypic antibodies.

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(21) International Application Number: PCT/US99/21677 (22) International Filing Date: 17 September 1999 (17.09.99) (30) Priority Data: 09/154,817 17 September 1998 (17.09.98) US (71) Applicant: ZYMOGENETICS, INC. [US/US]; 1201 Eastlake Avenue East, Seattle, WA 98102 (US). (72) Inventors: PRESNELL, Scott, R.; 2902 N. Puget Sound Avenue, Tacoma, WA 98407 (US). TAFT, David, W.; Apartment C, 264 E. Newton Street, Seattle, WA 98102 (US). FOLEY, Kevin, P.; 605 Boylston Avenue East #206, Seattle, WA 98102 (US). (74) Agent: LUNN, Paul, G.; ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seattle, WA 98102 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 25 May 2000 (25.05.00)
(54) Title: MAMMALIAN TRANSFORMING GROWTH FACTOR BETA - 9 (ZTGFSS9) (57) Abstract Novel mammalian Ztgfb-9 polypeptides, polynucleotides encoding the polypeptides, and related compositions and methods including antibodies and anti-idiotypic antibodies.		

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CLAIMS

We claim:

1. An isolated polynucleotide which encodes a polypeptide, said polypeptide being comprised of an amino acid sequence, said amino acid sequence being at least 90% identical to one or more of the amino acid sequences selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22.

2. An isolated polynucleotide of claim 1 wherein the polynucleotide encodes a polypeptide containing an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:17 and SEQ ID NO:18.

3. An isolated polynucleotide of claim 1 wherein the polynucleotide encodes a polypeptide comprised of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22.

4. An isolated polynucleotide of claim 3 wherein the polynucleotide encodes a polypeptide comprised of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5.

5. An isolated polynucleotide of claim 1 wherein said polynucleotide is selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:9.

6. An isolated polypeptide comprised of an amino acid sequence, said amino acid sequence being at least 90%

identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22.

7. An isolated polypeptide of claim 6 comprised of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22.

8. An isolated polypeptide of claim 6 wherein the amino acid sequence is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9 and SEQ ID NO:12.

9. An isolated polypeptide of claim 6 wherein the amino acid sequence is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5.

10. An antibody which specifically binds to a polypeptide comprised of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22.

11. An anti-idiotypic antibody which binds to an antibody of claim 12.

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MAMMALIAN TRANSFORMING GROWTH FACTOR BETA - 9

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BACKGROUND OF THE INVENTION

Proper control of the opposing processes of cell proliferation versus terminal differentiation and apoptotic programmed cell death is an important aspect of normal development and homeostasis, Raff, M.C., *Cell*, 86:173-175 (1996), and has been found to be altered in many human diseases. See, for example, Sawyers, C.L. et al., *Cell*, 64:337-350 (1991); Meyaard, L. et al., *Science*, 257:217-219 (1992); Guo, Q. et al., *Nature Med.*, 4:957-962 (1998); Barinaga, M. *Science*, 273:735-737 (1996); Solary, E. et al., *Eur. Respir. J.*, 9:1293-1305 (1996); Hamet, P. et al., *J. Hypertension*, 14:S65-S70, (1996); Roy, N. et al. *Cell*, 80:167-178 (1995); and Ambrosini, G., *Nature Med.*, 8:917-921 (1997). Much progress has been made towards understanding the regulation of this balance. For example, signaling cascades have been elucidated through which extracellular stimuli, such as growth factors, peptide hormones, and cell-cell interactions control the commitment of precursor cells to specific lineages and their subsequent proliferative expansion, Morrison, S.J. et al., *Cell*, 88:287-298 (1997). Further, it has been found that cell cycle exit and terminal differentiation are coupled in most cell types. See, for example, Coppola, J.A. et al. *Nature*, 320:760-763 (1986); Freytag, S.O., *Mol. Cell. Biol.* 8:1614-1624 (1988); Lee, E.Y. et al., *Genes Dev.*, 8:2008-2021 (1994); Morgenbesser, S.D., et al., *Nature*, 371:72-74 (1994); Casaccia-Bonnet, P. et al., *Genes Dev.*, 11:2335-2346 (1996); Zacksenhaus, E. et al., *Genes Dev.*, 10:3051-3064 (1996); and Zhang, P. et al., *Nature*, 387:151-158 (1997). Apoptosis (programmed cell death) also plays an important role in many developmental and homeostatic processes, Raff, M.C.,

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Nature, 356:397-400 (1992), and is often coordinately regulated with terminal differentiation, Jacobsen, K.A. et al., Blood, 84:2784-2794 (1994); Yan, Y. et al., Genes Dev., 11:973-983 (1997). Hence, it appears that the cell
5 type of individual lineages, tissues, organs, or even entire multicellular organisms is the result of a finely tuned balance between increased cell production due to proliferation, and decreased numbers of cells resulting from terminal differentiation and apoptosis. This balance
10 is most likely regulated coordinately by the convergence of multiple regulatory pathways. The identification of novel members of such networks can provide important insights into both normal cellular processes as well as the etiology and treatment of human disease states.

15

Interleukin 17 (IL-17) is a cytokine which has been implicated as an important regulator of the immune system, Spriggs, M.K., J. Clinical Immunology, 17:366-369 (1997), Broxmeyer, H.E., J. Experimental Medicine, 183:2411-2415
20 (1996), Yao, Z., et al., J. Immunology, 155:5483-5486 (1995), Yao, Z., et al., Immunity, 3:811-821 (1995). Human IL-17 is almost exclusively produced by activated CD4+ memory T cells (however, in mice, CD4-/CD8- T cells also express IL-17), Aarvak, T., et al., J. Immunology,
25 162:1246-1251 (1999), Kennedy, J., et al., J. Interferon Cytokine Research, 16:611-617 (1996). In contrast, the IL-17 receptor (IL-17R) appears to be ubiquitously expressed, Yao, Z., et al., Immunity, 3:811-821 (1995). IL-17 induces the secretion of IL-6, IL-8, monocyte chemotactic peptide-
30 1 and G-CSF from a variety of different stromal cell types, but has no effect on cytokine production by lymphoid cells, Teunissen, M.B.M., J. Investigative Dermatology, 111:645-649 (1998), Jovanovic, D.V., et al., J. Immunology, 160:3513-3521 (1998), Chabaud, M., et al.,
35 J. Immunology, 161:409-414 (1998), Cai, X.-Y., et al., Immunology Letters, 62:51-58 (1998), Fossiez, F., et al., J. Experimental Medicine, 183:2593-2603 (1996). IL-17

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also enhances the expression of ICAM-1 adhesion molecules on fibroblasts, and can stimulate granulopoiesis, Schwarzenberger P., et al., *J. Immunology*, 161:6383-9 (1998). . Taken together, these observations
5 have suggested that IL-17 functions as a pro-inflammatory cytokine. IL-17 also promotes dendritic cell differentiation, osteoclastogenesis, can induce nitric oxide production in human osteoarthritis cartilage, and is present in synovial fluids from patients with rheumatoid
10 arthritis, Antonysamy, M.A., et al., *J. Immunology*, 162:577-584 (1999), Kotake, S., et al., *J. Clinical Investigation*, 103:1345-1352, (1999), Attur, M.G., et al., *Arthritis & Rheumatism*, 40:1050-1053 (1997). Blocking IL-17 with a soluble IL-17R protein was found to suppress
15 cardiac allograft rejection, which correlated with increased IL-17 mRNA in kidney biopsies from humans undergoing renal allograft rejection, Antonysamy, M.A., et al., *J. Immunology*, 162:577-584 (1999). Increased IL-17 mRNA expression is also observed in humans with multiple
20 sclerosis, Matusevicius, D. et al., *Multiple Sclerosis*, 5:101-104 (1999). Further, IL-17 can promote tumorigenicity of human cervical tumors in nude mice, Tartour, E. et al., *Cancer Res.*, 59:3698-36704 (1999). Hence, IL-17 appears to play an essential role in
25 regulating the immune system and inflammatory processes.

Thus, there is a continuing need to discover new proteins involved with proliferation, differentiation, and
30 apoptotic pathways. The in vivo activities of both inducers and inhibitors of these pathways illustrates the enormous clinical potential of, and need for, novel proliferation, differentiation, and apoptotic proteins, their agonists and antagonists. There is also a need to
35 discover new agents which have anti-viral activity.

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SUMMARY OF THE INVENTION

The present invention addresses this need by providing a novel anti-viral polypeptide called
5 transforming growth factor beta-9, hereinafter referred to as Ztgf β -9, and related compositions and methods. This polypeptide has anti-viral activity as disclosed in Example 10 below. It may also be used to regulate the proliferation, differentiation and apoptosis of neurons
10 glial cells, lymphocytes, hematopoietic cells and stromal cells.

Thus, one aspect of the present invention provides for an isolated Ztgf β -9 polypeptide and
15 polynucleotide. The human sequences are defined by SEQ ID NOs: 1 and 2.

The nucleotide sequence of SEQ ID NO:1 contains an open reading frame encoding a polypeptide of about 202
20 amino acids with the initial Met as shown in SEQ ID NO:1 and SEQ ID NO:2. A predicted signal sequence is comprised of amino acid residues 1, a methionine extending to and includes amino acid residue 15, an alanine. Thus a mature sequence excluding the signal sequence extends from amino
25 acid residue 16, an alanine, to and including amino acid residue 202 a proline, of SEQ ID NO:2. This mature sequence is also represented by SEQ ID NO:3. In an alternative embodiment the signal sequence extends to and includes amino acid residue 16, an alanine. This produces
30 a mature sequence which extends from amino acid 17, a glycine, to and including amino acid residue 202, a proline, of SEQ ID NO:2. This mature sequence is also represented by SEQ ID NO:4. In another alternative embodiment, the signal sequence extends to and includes
35 amino acid residue 17, a glycine. This results in a mature sequence which extends from amino acid residue 18, an alanine, to and including amino acid residue 202, a

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proline, of SEQ ID NO:2. This mature sequence is further represented by SEQ ID NO:5. Another variant of Ztgfb-9 is disclosed by SEQ ID NOs: 16 and 17. The mature sequence extends from amino acid residue 23, an alanine, to and including amino acid residue 209, a proline. The mature sequence is also defined by SEQ ID NO:18.

Murine Ztgfb-9 is defined by SEQ ID NOs: 8 and 9. The signal sequence extends from the methionine at position 1 through the alanine at position 22. Thus the mature sequence extends from the alanine at position 23 of SEQ ID NO:9 through the arginine at position 205. The mature sequence is further represented by SEQ ID NO:12.

An additional embodiment of the present invention relates to a peptide or polypeptide which has the amino acid sequence of an epitope-bearing portion of a Ztgfb-9 polypeptide having an amino acid sequence described above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a Ztgfb-9 polypeptide of the present invention include portions of such polypeptides with at least nine, preferably at least 15 and more preferably at least 30 to 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the present invention described above are also included in the present invention. Examples of such epitope-bearing polypeptides are SEQ ID NOs: 13, 14, 15, 19, 20, 21 and 22. Also claimed are any of these polypeptides that are fused to another polypeptide or carrier molecule. Also claimed is an isolated nucleic acid which encodes an epitope-bearing portion of a Ztgfb-9 polypeptide.

The present invention is further comprised of an isolated peptide or polypeptide of the above-described peptides or polypeptides having an amino acid sequence

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modified by addition, deletion and/or replacement of one or more amino acid residues and which maintains the biological activity of said peptide or polypeptide.

5 Within a further aspect of the invention there is provided a chimeric polypeptide consisting essentially of a first portion and a second portion joined by a peptide bond. The first portion of the chimeric polypeptide consists essentially of (a) a Ztgf β -9
10 polypeptide as described above (b) allelic variants of the polypeptides described above. The second portion of the chimeric polypeptide consists essentially of another polypeptide such as an affinity tag. Within one
embodiment the affinity tag is an immunoglobulin F_C
15 polypeptide. The invention also provides expression vectors encoding the chimeric polypeptides and host cells transfected to produce the chimeric polypeptides.

Another aspect of the present invention provides
20 for isolated nucleic acid molecules comprising a polynucleotide selected from the group consisting of: (a) a nucleotide sequence encoding the Ztgf β -9 polypeptides described above; and (b) a nucleotide sequence
complementary to any of the nucleotide sequences in (a).

25

Further embodiments of the invention include isolated nucleic acid molecules that comprise a polynucleotide having a nucleotide sequence at least 90% homologous, and more preferably 95%, 97%, 98%, or 99%
30 homologous to any of the nucleotide sequences in (a) or (b) above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence of (a) or (b) above.

35

Further embodiments of the invention include isolated polypeptides having an amino acid sequence that is at least 90% identical, and more preferably 95%, 97%,

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98%, or 99% identical to any of the Ztgf β -9 polypeptides and polynucleotides which encode these polypeptides.

Within another aspect of the invention there is provided an expression vector comprising (a) a transcription promoter; (b) a DNA segment encoding a polypeptide described above, and (c) a transcription terminator, wherein the promoter, DNA segment, and terminator are operably linked.

10

Within a third aspect of the invention there is provided a cultured eukaryotic cell into which has been introduced an expression vector as disclosed above, wherein said cell expresses a protein polypeptide encoded by the DNA segment.

15

In another embodiment of the present invention is an isolated antibody that binds specifically to a Ztgf β -9 polypeptide described above. Also claimed is a method for producing antibodies which bind to a Ztgf β -9 polypeptide comprising inoculating a mammal with a Ztgf β -9 polypeptide or Ztgf β -9 epitope-bearing polypeptide so that the mammal produces antibodies to the polypeptide; and isolating said antibodies.

20

These and other aspects of the invention will become evident upon reference to the following detailed description.

30 DETAILED DESCRIPTION OF THE INVENTION

The teachings of all of the references cited herein are incorporated in their entirety herein by reference.

35

In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

5 As used herein, "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of
10 ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., α -enantiomeric forms of naturally-
15 occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups,
20 amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety
25 include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate,
30 phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term "nucleic acid molecule" also includes so-called "peptide nucleic acids," which comprise naturally-
35 occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

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The term "complement of a nucleic acid molecule" refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a nucleic acid molecule that has a contiguous stretch of identical or complementary sequence to another nucleic acid molecule. Contiguous sequences are said to "overlap" a given stretch of a nucleic acid molecule either in their entirety or along a partial stretch of the nucleic acid molecule.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

The term "structural gene" refers to a nucleic acid molecule that is transcribed into messenger RNA (mRNA), which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than

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the complete DNA molecule of a chromosome from that species.

A "nucleic acid molecule construct" is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature.

10 "Linear DNA" denotes non-circular DNA molecules having free 5' and 3' ends. Linear DNA can be prepared from closed circular DNA molecules, such as plasmids, by enzymatic digestion or physical disruption.

15 "Complementary DNA (cDNA)" is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term "cDNA" also refers to a clone of a cDNA molecule synthesized from an RNA template.

25 A "promoter" is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee et al., *Mol. Endocrinol.* 7:551 (1993)), cyclic AMP response elements (CREs), serum response elements (SREs; Treisman, *Seminars in Cancer*

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Biol. 1:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly et al., *J. Biol. Chem.* 267:19938 (1992)), AP2 (Ye et al., *J. Biol. Chem.* 269:25728 (1994)),
5 SP1, cAMP response element binding protein (CREB; Loeken, *Gene Expr.* 3:253 (1993)) and octamer factors (see, in general, Watson et al., eds., *Molecular Biology of the Gene*, 4th ed. (The Benjamin/Cummings Publishing Company, Inc. 1987), and Lemaigre and Rousseau, *Biochem. J.* 303:1
10 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also
15 known.

A "core promoter" contains essential nucleotide sequences for promoter function, including the TATA box and start of transcription. By this definition, a core
20 promoter may or may not have detectable activity in the absence of specific sequences that may enhance the activity or confer tissue specific activity.

A "regulatory element" is a nucleotide sequence
25 that modulates the activity of a core promoter. For example, a regulatory element may contain a nucleotide sequence that binds with cellular factors enabling transcription exclusively or preferentially in particular cells, tissues, or organelles. These types of regulatory
30 elements are normally associated with genes that are expressed in a "cell-specific," "tissue-specific," or "organelle-specific" manner. For example, the Ztgf β -9 regulatory element preferentially induces gene expression in brain, spinal cord, heart, skeletal muscle, stomach,
35 pancreas, adrenal gland, salivary gland, liver, small intestine, bone marrow, thymus, spleen, lymph node, heart, thyroid, trachea, testis, ovary and placenta.

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An "enhancer" is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

"Heterologous DNA" refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (i.e., endogenous DNA) so long as that host DNA is combined with non-host DNA (i.e., exogenous DNA). For example, a DNA molecule containing a non-host DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule. Conversely, a heterologous DNA molecule can comprise an endogenous gene operably linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

30

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone

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structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

A peptide or polypeptide encoded by a non-host
5 DNA molecule is a "heterologous" peptide or polypeptide.

An "integrated genetic element" is a segment of DNA that has been incorporated into a chromosome of a host cell after that element is introduced into the cell
10 through human manipulation. Within the present invention, integrated genetic elements are most commonly derived from linearized plasmids that are introduced into the cells by electroporation or other techniques. Integrated genetic elements are passed from the original host cell to its
15 progeny.

A "cloning vector" is a nucleic acid molecule, such as a plasmid, cosmid, or bacteriophage, that has the capability of replicating autonomously in a host cell.
20 Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites that allow insertion of a nucleic acid molecule in a determinable fashion without loss of an essential biological function of the vector, as well as nucleotide sequences encoding a
25 marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

30 An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a
35 promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a

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core promoter are operably linked if the regulatory element modulates the activity of the core promoter.

A "recombinant host" is a cell that contains a
5 heterologous nucleic acid molecule, such as a cloning
vector or expression vector. In the present context, an
example of a recombinant host is a cell that produces
Ztgf β -9 from an expression vector. In contrast, Ztgf β -9
can be produced by a cell that is a "natural source" of
10 Ztgf β -9, and that lacks an expression vector.

"Integrative transformants" are recombinant host
cells, in which heterologous DNA has become integrated
into the genomic DNA of the cells.

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A "fusion protein" is a hybrid protein expressed
by a nucleic acid molecule comprising nucleotide sequences
of at least two genes. For example, a fusion protein can
comprise at least part of a Ztgf β -9 polypeptide fused with
20 a polypeptide that binds an affinity matrix. Such a
fusion protein provides a means to isolate large
quantities of Ztgf β -9 using affinity chromatography.

The term "receptor" denotes a cell-associated
25 protein that binds to a bioactive molecule termed a
"ligand." This interaction mediates the effect of the
ligand on the cell. Receptors can be membrane bound,
cytosolic or nuclear; monomeric (e.g., thyroid stimulating
hormone receptor, beta-adrenergic receptor) or multimeric
30 (e.g., PDGF receptor, growth hormone receptor, IL-3
receptor, GM-CSF receptor, G-CSF receptor, erythropoietin
receptor and IL-6 receptor). Membrane-bound receptors are
characterized by a multi-domain structure comprising an
extracellular ligand-binding domain and an intracellular
35 effector domain that is typically involved in signal
transduction. In certain membrane-bound receptors, the
extracellular ligand-binding domain and the intracellular

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effector domain are located in separate polypeptides that comprise the complete functional receptor.

In general, the binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell, which in turn leads to an alteration in the metabolism of the cell. Metabolic events that are often linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids.

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The term "secretory signal sequence" denotes a DNA sequence that encodes a peptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

An "isolated polypeptide" is a polypeptide that is essentially free from contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated polypeptide contains the polypeptide in a highly purified form, i.e., at least about 80% pure, at least about 90% pure, at least about 95% pure, greater than 95% pure, or greater than 99% pure. One way to show that a particular protein preparation contains an isolated polypeptide is by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. However, the term "isolated" does not exclude

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the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

5 The terms "amino-terminal or N-terminal" and "carboxyl-terminal or C-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote
10 proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete
15 polypeptide.

 The term "expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the
20 structural gene into mRNA and the translation of mRNA into one or more polypeptides.

 The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene.
25 Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode
30 polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a polypeptide encoded by a splice variant of an mRNA transcribed from a gene.

35 As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors,

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lymphotoxins, co-stimulatory molecules, hematopoietic factors, and synthetic analogs of these molecules.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of less than 10^9 M^{-1} .

An "anti-idiotypic antibody" is an antibody that binds with the variable region domain of an immunoglobulin. In the present context, an anti-idiotypic antibody binds with the variable region of an anti-Ztgf β -9 antibody, and thus, an anti-idiotypic antibody mimics an epitope of Ztgf β -9.

An "antibody fragment" is a portion of an antibody such as F(ab')_2 , F(ab)_2 , Fab' , Fab , and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. For example, a Ztgf β -9 monoclonal antibody fragment binds with an epitope of Ztgf β -9.

30

The term "antibody fragment" also includes a synthetic or a genetically engineered polypeptide that binds to a specific antigen, such as polypeptides consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are

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connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

5 A "chimeric antibody" is a recombinant protein that contains the variable domains and complementary determining regions derived from a rodent antibody, while the remainder of the antibody molecule is derived from a human antibody.

10 "Humanized antibodies" are recombinant proteins in which murine complementarity determining regions of a monoclonal antibody have been transferred from heavy and light variable chains of the murine immunoglobulin into a
15 human variable domain.

 As used herein, a "therapeutic agent" is a molecule or atom which is conjugated to an antibody moiety to produce a conjugate which is useful for therapy.
20 Examples of therapeutic agents include drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes.

 A "detectable label" is a molecule or atom which
25 can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties.

30 The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of
35 the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag.

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Affinity tags include a poly-histidine tract, protein A (Nilsson et al., *EMBO J.* 4:1075 (1985); Nilsson et al., *Methods Enzymol.* 198:3 (1991)), glutathione S transferase (Smith and Johnson, *Gene* 67:31 (1988)), Glu-Glu affinity tag (Grussenmeyer et al., *Proc. Natl. Acad. Sci. USA* 82:7952 (1985)), substance P, FLAG peptide (Hopp et al., *Biotechnology* 6:1204 (1988)), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., *Protein Expression and Purification* 2:95 (1991). DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

A "naked antibody" is an entire antibody, as opposed to an antibody fragment, which is not conjugated with a therapeutic agent. Naked antibodies include both polyclonal and monoclonal antibodies, as well as certain recombinant antibodies, such as chimeric and humanized antibodies.

20

As used herein, the term "antibody component" includes both an entire antibody and an antibody fragment.

An "immunoconjugate" is a conjugate of an antibody component with a therapeutic agent or a detectable label.

As used herein, the term "antibody fusion protein" refers to a recombinant molecule that comprises an antibody component and a therapeutic agent. Examples of therapeutic agents suitable for such fusion proteins include immunomodulators ("antibody-immunomodulator fusion protein") and toxins ("antibody-toxin fusion protein").

A "tumor associated antigen" is a protein normally not expressed, or expressed at lower levels, by a normal counterpart cell. Examples of tumor associated

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antigens include alpha-fetoprotein, carcinoembryonic antigen, and Her-2/neu. Many other illustrations of tumor associated antigens are known to those of skill in the art. See, for example, Urban et al., *Ann. Rev. Immunol.* 5 10:617 (1992).

As used herein, an "infectious agent" denotes both microbes and parasites. A "microbe" includes viruses, bacteria, rickettsia, mycoplasma, protozoa, fungi 10 and like microorganisms. A "parasite" denotes infectious, generally microscopic or very small multicellular invertebrates, or ova or juvenile forms thereof, which are susceptible to immune-mediated clearance or lytic or phagocytic destruction, such as malarial parasites, 15 spirochetes, and the like.

An "infectious agent antigen" is an antigen associated with an infectious agent.

20 A "target polypeptide" or a "target peptide" is an amino acid sequence that comprises at least one epitope, and that is expressed on a target cell, such as a tumor cell, or a cell that carries an infectious agent antigen. T cells recognize peptide epitopes presented by 25 a major histocompatibility complex molecule to a target polypeptide or target peptide and typically lyse the target cell or recruit other immune cells to the site of the target cell, thereby killing the target cell.

30 An "antigenic peptide" is a peptide which will bind a major histocompatibility complex molecule to form an MHC-peptide complex which is recognized by a T cell, thereby inducing a cytotoxic lymphocyte response upon presentation to the T cell. Thus, antigenic peptides are 35 capable of binding to an appropriate major histocompatibility complex molecule and inducing a cytotoxic T cells response, such as cell lysis or specific

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cytokine release against the target cell which binds or expresses the antigen. The antigenic peptide can be bound in the context of a class I or class II major histocompatibility complex molecule, on an antigen
5 presenting cell or on a target cell.

In eukaryotes, RNA polymerase II catalyzes the transcription of a structural gene to produce mRNA. A nucleic acid molecule can be designed to contain an RNA
10 polymerase II template in which the RNA transcript has a sequence that is complementary to that of a specific mRNA. The RNA transcript is termed an "anti-sense RNA" and a nucleic acid molecule that encodes the anti-sense RNA is termed an "anti-sense gene." Anti-sense RNA molecules are
15 capable of binding to mRNA molecules, resulting in an inhibition of mRNA translation or mRNA degradation.

An "anti-sense oligonucleotide specific for *Ztgf β -9*" or a "*Ztgf β -9* anti-sense oligonucleotide" is an
20 oligonucleotide having a sequence (a) capable of forming a stable triplex with a portion of the *Ztgf β -9* gene, or (b) capable of forming a stable duplex with a portion of an mRNA transcript of the *Ztgf β -9* gene.

A "ribozyme" is a nucleic acid molecule that contains a catalytic center. The term includes RNA enzymes, self-splicing RNAs, self-cleaving RNAs, and nucleic acid molecules that perform these catalytic functions. A nucleic acid molecule that encodes a
30 ribozyme is termed a "ribozyme gene."

An "external guide sequence" is a nucleic acid molecule that directs the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, resulting in
35 the cleavage of the mRNA by RNase P. A nucleic acid molecule that encodes an external guide sequence is termed an "external guide sequence gene."

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The term "variant human *Ztgfβ-9* gene" refers to nucleic acid molecules that encode a polypeptide having an amino acid sequence that is a modification of SEQ ID NO:2. Such variants include naturally-occurring polymorphisms of *Ztgfβ-9* genes, as well as synthetic genes that contain conservative amino acid substitutions of the amino acid sequence of SEQ ID NO:2. Additional variant forms of *Ztgfβ-9* genes are nucleic acid molecules that contain insertions or deletions of the nucleotide sequences described herein. A variant *Ztgfβ-9* gene can be identified by determining whether the gene hybridizes with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, or its complement, under stringent conditions.

Similarly, the term "variant murine *Ztgfβ-9* gene" refers to nucleic acid molecules that encode a polypeptide having an amino acid sequence that is a modification of SEQ ID NO:9. A variant murine *Ztgfβ-9* gene can be identified by determining whether the gene hybridizes with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:8, or its complement, under stringent conditions.

Alternatively, variant *Ztgfβ-9* genes can be identified by sequence comparison. Two amino acid sequences have "100% amino acid sequence identity" if the amino acid residues of the two amino acid sequences are the same when aligned for maximal correspondence. Similarly, two nucleotide sequences have "100% nucleotide sequence identity" if the nucleotide residues of the two nucleotide sequences are the same when aligned for maximal correspondence. Sequence comparisons can be performed using standard software programs such as those included in the LASERGENE bioinformatics computing suite, which is produced by DNASTAR (Madison, Wisconsin). Other methods

for comparing two nucleotide or amino acid sequences by determining optimal alignment are well-known to those of skill in the art (see, for example, Peruski and Peruski, *The Internet and the New Biology: Tools for Genomic and*
5 *Molecular Research* (ASM Press, Inc. 1997), Wu et al. (eds.), "Information Superhighway and Computer Databases of Nucleic Acids and Proteins," in *Methods in Gene Biotechnology*, pages 123-151 (CRC Press, Inc. 1997), and Bishop (ed.), *Guide to Human Genome Computing*, 2nd Edition
10 (Academic Press, Inc. 1998)). Particular methods for determining sequence identity are described below.

Regardless of the particular method used to identify a variant *Ztgf β -9* gene or variant *Ztgf β -9*
15 polypeptide, a variant gene or polypeptide encoded by a variant gene is functionally characterized by either its anti-viral or anti-proliferative activities, or by the ability to bind specifically to an anti-*Ztgf β -9* antibody.

20 The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene
25 mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

30 The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the
35 result of speciation.

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"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α -globin, β -globin, and myoglobin are paralogs of each other.

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Due to the imprecision of standard analytical methods, molecular weights and lengths of polymers are understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated
10 value of X will be understood to be accurate to $\pm 10\%$.

Nucleic acid molecules encoding a human or mouse *Ztgf β -9* gene can be obtained by screening a human or mouse cDNA or genomic library using polynucleotide probes based
15 upon SEQ ID NO:1 or SEQ ID NO:8. These techniques are standard and well-established. As an illustration, a nucleic acid molecule that encodes a human *Ztgf β -9* gene can be isolated from a human cDNA library. In this case, the first step would be to prepare the cDNA library by
20 isolating RNA from brain, spinal cord, heart, skeletal muscle, stomach, pancreas, adrenal gland, salivary gland, liver, small intestine, bone marrow, thymus, spleen, lymph node, heart, thyroid, trachea, testis, ovary or placental tissue, using methods well-known to those of skill in the
25 art. In general, RNA isolation techniques must provide a method for breaking cells, a means of inhibiting RNase-directed degradation of RNA, and a method of separating RNA from DNA, protein, and polysaccharide contaminants. For example, total RNA can be isolated by freezing tissue in
30 liquid nitrogen, grinding the frozen tissue with a mortar and pestle to lyse the cells, extracting the ground tissue with a solution of phenol/chloroform to remove proteins, and separating RNA from the remaining impurities by selective precipitation with lithium chloride (see, for
35 example, Ausubel et al. (eds.), *Short Protocols in Molecular Biology*, 3rd Edition, pages 4-1 to 4-6 (John Wiley & Sons 1995) ["Ausubel (1995)"]; Wu et al., *Methods in Gene*

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Biotechnology, pages 33-41 (CRC Press, Inc. 1997) ["Wu (1997)"]).

Alternatively, total RNA can be isolated from
5 brain or spinal cord tissue as well as heart, skeletal
muscle, stomach, pancreas, adrenal gland, salivary gland,
liver, small intestine, bone marrow, thymus, spleen, lymph
node, thyroid, trachea, testis, ovary or placenta by
extracting ground tissue with guanidinium isothiocyanate,
10 extracting with organic solvents, and separating RNA from
contaminants using differential centrifugation (see, for
example, Chirgwin et al., *Biochemistry* 18:52 (1979);
Ausubel (1995) at pages 4-1 to 4-6; Wu (1997) at pages 33-
41). To construct a cDNA library, poly(A)⁺ RNA must be
15 isolated from a total RNA preparation. Poly(A)⁺ RNA can be
isolated from total RNA using the standard technique of
oligo(dT)-cellulose chromatography (see, for example, Aviv
and Leder, *Proc. Nat'l Acad. Sci. USA* 69:1408 (1972);
Ausubel (1995) at pages 4-11 to 4-12). Double-stranded cDNA
20 molecules are synthesized from poly(A)⁺ RNA using
techniques well-known to those in the art. (see, for
example, Wu (1997) at pages 41-46). Moreover,
commercially available kits can be used to synthesize
double-stranded cDNA molecules. For example, such kits
25 are available from Life Technologies, Inc. (Gaithersburg,
MD), CLONTECH Laboratories, Inc. (Palo Alto, CA), Promega
Corporation (Madison, WI) and STRATAGENE (La Jolla, CA).

Various cloning vectors are appropriate for the
30 construction of a cDNA library. For example, a cDNA
library can be prepared in a vector derived from
bacteriophage, such as a λ gt10 vector. See, for example,
Huynh et al., "Constructing and Screening cDNA Libraries
in λ gt10 and λ gt11," in *DNA Cloning: A Practical Approach*
35 Vol. I, Glover (ed.), page 49 (IRL Press, 1985); Wu (1997)
at pages 47-52. Alternatively, double-stranded cDNA
molecules can be inserted into a plasmid vector, such as a

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pBLUESCRIPT vector (STRATAGENE; La Jolla, CA), a LAMDA GEM-4 (Promega Corp.) or other commercially available vectors. Suitable cloning vectors also can be obtained from the American Type Culture Collection (Manassas, VA). To amplify
5 the cloned cDNA molecules, the cDNA library is inserted into a prokaryotic host, using standard techniques. For example, a cDNA library can be introduced into competent *E. coli* DH5 cells, which can be obtained, for example, from Life Technologies, Inc. (Gaithersburg, MD).

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A human genomic library can be prepared by means well-known in the art (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327). Genomic DNA can be isolated by lysing tissue with the detergent
15 Sarkosyl, digesting the lysate with proteinase K, clearing insoluble debris from the lysate by centrifugation, precipitating nucleic acid from the lysate using isopropanol, and purifying resuspended DNA on a cesium chloride density gradient. DNA fragments that are suitable
20 for the production of a genomic library can be obtained by the random shearing of genomic DNA or by the partial digestion of genomic DNA with restriction endonucleases. Genomic DNA fragments can be inserted into a vector, such as a bacteriophage or cosmid vector, in accordance with
25 conventional techniques, such as the use of restriction enzyme digestion to provide appropriate termini, the use of alkaline phosphatase treatment to avoid undesirable joining of DNA molecules, and ligation with appropriate ligases. Techniques for such manipulation are well-known in the art
30 (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327).

Nucleic acid molecules that encode a human *Ztgf β -9* gene can also be obtained using the polymerase
35 chain reaction (PCR) with oligonucleotide primers having nucleotide sequences that are based upon the nucleotide sequences of the human *Ztgf β -9* gene, as described herein.

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General methods for screening libraries with PCR are provided by, for example, Yu et al., "Use of the Polymerase Chain Reaction to Screen Phage Libraries," in *Methods in Molecular Biology*, Vol. 15: *PCR Protocols: Current Methods and Applications*, White (ed.), pages 211-215 (Humana Press, Inc. 1993). Moreover, techniques for using PCR to isolate related genes are described by, for example, Preston, "Use of Degenerate Oligonucleotide Primers and the Polymerase Chain Reaction to Clone Gene Family Members," in *Methods in Molecular Biology*, Vol. 15: *PCR Protocols: Current Methods and Applications*, White (ed.), pages 317-337 (Humana Press, Inc. 1993). Alternatively, human genomic libraries can be obtained from commercial sources such as Research Genetics (Huntsville, AL) and the American Type Culture Collection (Manassas, VA). A library containing cDNA or genomic clones can be screened with one or more polynucleotide probes based upon SEQ ID NO:1, using standard methods (see, for example, Ausubel (1995) at pages 6-1 to 6-11).

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Anti- Ztgf β -9 antibodies, produced as described below, can also be used to isolate DNA sequences that encode human Ztgf β -9 genes from cDNA libraries. For example, the antibodies can be used to screen λ gt11 expression libraries, or the antibodies can be used for immunoscreening following hybrid selection and translation (see, for example, Ausubel (1995) at pages 6-12 to 6-16; Margolis et al., "Screening λ expression libraries with antibody and protein probes," in *DNA Cloning 2: Expression Systems*, 2nd Edition, Glover et al. (eds.), pages 1-14 (Oxford University Press 1995)).

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As an alternative, an Ztgf β -9 gene can be obtained by synthesizing nucleic acid molecules using mutually priming long oligonucleotides and the nucleotide sequences described herein (see, for example, Ausubel (1995) at pages 8-8 to 8-9). Established techniques using

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the polymerase chain reaction provide the ability to synthesize DNA molecules at least two kilobases in length (Adang et al., *Plant Molec. Biol.* 21:1131 (1993), Bambot et al., *PCR Methods and Applications* 2:266 (1993), Dillon
5 et al., "Use of the Polymerase Chain Reaction for the Rapid Construction of Synthetic Genes," in *Methods in Molecular Biology*, Vol. 15: *PCR Protocols: Current Methods and Applications*, White (ed.), pages 263-268, (Humana Press, Inc. 1993), and Holowachuk et al., *PCR Methods*
10 *Appl.* 4:299 (1995)). The sequence of an *Ztgfβ-9* cDNA or *Ztgfβ-9* genomic fragment can be determined using standard methods. Moreover, the identification of genomic fragments containing an *Ztgfβ-9* promoter or regulatory element can be achieved using well-established techniques,
15 such as deletion analysis (see, generally, Ausubel (1995)).

Cloning of 5' flanking sequences also facilitates production of *Ztgfβ-9* proteins by "gene
20 activation," following the methods disclosed in U.S. Patent No. 5,641,670. Briefly, expression of an endogenous *Ztgfβ-9* gene in a cell is altered by introducing into the *Ztgfβ-9* locus a DNA construct comprising at least a targeting sequence, a regulatory sequence, an exon, and
25 an unpaired splice donor site. The targeting sequence is a *Ztgfβ-9* 5' non-coding sequence that permits homologous recombination of the construct with the endogenous *Ztgfβ-9* locus, whereby the sequences within the construct become operably linked with the endogenous *Ztgfβ-9* coding
30 sequence. In this way, an endogenous *Ztgfβ-9* promoter can be replaced or supplemented with other regulatory sequences to provide enhanced, tissue-specific, or otherwise regulated expression.

35 Additionally, the polynucleotides of the present invention can be synthesized using a DNA synthesizer. Currently the method of choice is the phosphoramidite

method. If chemically synthesized double stranded DNA is required for an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short genes (60 to 80
5 bp) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes (>300 bp), however, special strategies must be invoked, because the coupling efficiency of each cycle during chemical DNA
10 synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length. In addition to the protein coding sequence, synthetic genes can be designed with
15 terminal sequences that facilitate insertion into a restriction endonuclease sites of a cloning vector and other sequences should also be added that contain signals for the proper initiation and termination of transcription and translation. See Glick, Bernard R. and Jack J.
20 Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA*, (ASM Press, Washington, D.C. 1994), Itakura, K. et al. Synthesis and use of synthetic oligonucleotides. *Annu. Rev. Biochem.* 53 : 323-356 (1984), and Climie, S. et al. Chemical synthesis of
25 the thymidylate synthase gene. *Proc. Natl. Acad. Sci. USA* 87 :633-637 (1990).

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar
30 sized regions of the DNA of SEQ ID NO:1, or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is
35 the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typical stringent conditions are those in

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which the salt concentration is about 0.02 M or less at pH 7 and the temperature is at least about 60°C. As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. Total RNA can be prepared using guanidine HCl extraction followed by isolation by centrifugation in a CsCl gradient [Chirgwin et al., *Biochemistry* 18:52-94 (1979)]. Poly (A)⁺ RNA is prepared from total RNA using the method of Aviv and Leder, *Proc. Natl. Acad. Sci. USA* 69:1408-1412 (1972). Complementary DNA (cDNA) is prepared from poly(A)⁺ RNA using known methods. Polynucleotides encoding Ztgfβ-9 polypeptides are then identified and isolated by, for example, hybridization or PCR.

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Those skilled in the art will recognize that the sequences disclosed in SEQ ID NOS:1 and 2 represent a single allele of the human. There are a number of naturally occurring mature N-terminal variants having the leader sequence cleaved at differing positions. Allelic variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. The present invention further provides counterpart proteins and polynucleotides from other species ("species orthologs"). Of particular interest are Ztgfβ-9 polypeptides from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine, and other primates. Species orthologs of the human Ztgfβ-9 protein can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the gene. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a

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positive tissue or cell line. A protein-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent No. 4,683,202), using primers designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to the protein. Similar techniques can also be applied to the isolation of genomic clones. As used and claimed the language "an isolated polynucleotide which encodes a polypeptide, said polynucleotide being defined by SEQ ID NO: 2" includes all allelic variants and species orthologs of the polypeptide of SEQ ID NOS:2, 3, 4 and 5.

Within preferred embodiments of the invention, isolated nucleic acid molecules that encode human Ztgf β -9 can hybridize to nucleic acid molecules having the nucleotide sequence of SEQ ID NO:1, or a sequence complementary thereto, under "stringent conditions." In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.

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As an illustration, a nucleic acid molecule encoding a variant Ztgf β -9 polypeptide can be hybridized with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement) at 42°C overnight in a solution comprising 50% formamide, 5xSSC (1xSSC: 0.15 M sodium chloride and 15 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution

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(100x Denhardt's solution: 2% (w/v) Ficoll 400, 2% (w/v) polyvinylpyrrolidone, and 2% (w/v) bovine serum albumin), 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA. One of skill in the art can devise variations of these hybridization conditions. For example, the hybridization mixture can be incubated at a higher temperature, such as about 65°C, in a solution that does not contain formamide. Moreover, premixed hybridization solutions are available (e.g., EXPRESSHYB Hybridization Solution from CLONTECH Laboratories, Inc.), and hybridization can be performed according to the manufacturer's instructions. Following hybridization, the nucleic acid molecules can be washed to remove non-hybridized nucleic acid molecules under stringent conditions, or under highly stringent conditions. Typical stringent washing conditions include washing in a solution of 0.5x - 2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 55 - 65°C. That is, nucleic acid molecules encoding a variant Ztgfβ-9 polypeptide hybridize with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C, including 0.5x SSC with 0.1% SDS at 55°C, or 2xSSC with 0.1% SDS at 65°C. One of skill in the art can readily devise equivalent conditions, for example, by substituting SSPE for SSC in the wash solution.

Typical highly stringent washing conditions include washing in a solution of 0.1x - 0.2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 50 - 65°C. In other words, nucleic acid molecules encoding a variant Ztgfβ-9 polypeptide hybridize with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1%

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SDS at 50 - 65°C, including 0.1x SSC with 0.1% SDS at 50°C, or 0.2xSSC with 0.1% SDS at 65°C.

The present invention also provides isolated
5 Ztgf β -9 polypeptides that have a substantially similar
sequence identity to the polypeptides of SEQ ID NOs:2,3,
4, 5, 9, 12, 17, 18 or their orthologs. The term
"substantially similar sequence identity" is used herein
to denote polypeptides having at least 70%, at least 80%,
10 at least 90%, at least 95% or greater than 95% and 99%
sequence identity to the sequences shown in SEQ ID NOs:2,
3, 4, 5, 9, 12, 17, 18 or their orthologs.

The present invention also contemplates Ztgf β -9
15 variant nucleic acid molecules that can be identified
using two criteria: a determination of the similarity
between the encoded polypeptide with the amino acid
sequence of SEQ ID NOs:2, 3, 4, 5, 9, 12, 17 or 18, and a
hybridization assay, as described above. Such Ztgf β -9
20 variants include nucleic acid molecules (1) that hybridize
with a nucleic acid molecule having the nucleotide
sequence of SEQ ID NO:1, SEQ ID NO: 9 or SEQ ID NO:16 (or
their complement) under stringent washing conditions, in
which the wash stringency is equivalent to 0.5x - 2x SSC
25 with 0.1% SDS at 55 - 65°C, and (2) that encode a
polypeptide having at least 70%, at least 80%, at least
90%, at least 95% or greater than 95% sequence identity to
the amino acid sequence of SEQ ID NOs:2, 3, 4, 5, 9, 12,
17 or 18. Alternatively, Ztgf β -9 variants can be
30 characterized as nucleic acid molecules (1) that hybridize
with a nucleic acid molecule having the nucleotide
sequence of SEQ ID NO:1 (or its complement) under highly
stringent washing conditions, in which the wash stringency
is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 -
35 65°C, and (2) that encode a polypeptide having at least
70%, at least 80%, at least 90%, at least 95% or greater

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than 95% or 99% sequence identity to the amino acid sequence of SEQ ID NOs:2, 3, 4, 5, 9, 12, 17 or 18.

The present invention also contemplates human
5 *Ztgfβ-9* variant nucleic acid molecules identified by at least one of hybridization analysis and sequence identity determination, with reference to SEQ ID NO:2. The present invention further includes murine *Ztgfβ-9* variant nucleic acid molecules identified by at least one of hybridization
10 analysis and sequence identity determination, with reference to SEQ ID NOs:8 and 9. For example, using the approach discussed above, murine *Ztgfβ-9* variant nucleic acid molecules can be identified using at least one of three criteria: (1) hybridization with a nucleic acid
15 molecule having the nucleotide sequence of SEQ ID NO:8 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C, (2) hybridization with a nucleic acid molecule having the nucleotide sequence of
20 SEQ ID NO:8 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C, and (3) an amino acid percent identity that is at least 70%, at least 80%, at least 90%, at least 95% or greater
25 than 95% sequence identity to the amino acid sequence of SEQ ID NO:12.

Percent sequence identity is determined by conventional methods. See, for example, Altschul et al.,
30 *Bull. Math. Bio.* 48:603 (1986), and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM 62" scoring matrix of
35 Henikoff and Henikoff (*ibid.*) as shown in Table 1 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as: ([Total number

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of identical matches]/ [length of the longer sequence plus
the number of gaps introduced into the longer sequence in
order to align the two sequences]) (100).

Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative Ztgf β -9 variant. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA* 85:2444 (1988), and by Pearson, *Meth. Enzymol.* 183:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (e.g., SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then re-scored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol.* 48:444 (1970); Sellers, *SIAM J. Appl. Math.* 26:787 (1974)), which allows for amino acid insertions and deletions. Illustrative parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file

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("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol.* 183:63 (1990).

FASTA can also be used to determine the sequence
5 identity of nucleic acid molecules using a ratio as
disclosed above. For nucleotide sequence comparisons, the
ktup value can range between one to six, preferably from
three to six, most preferably three, with other parameters
set as described above.

10

The present invention includes nucleic acid
molecules that encode a polypeptide having a conservative
amino acid change, compared with the amino acid sequence
of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO:5,
15 SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:17 or SEQ ID NO:18.
That is, variants can be obtained that contain one or more
amino acid substitutions of SEQ ID NO:2, SEQ ID NO:3, SEQ
ID NO: 4 SEQ ID NO:5 SEQ ID NO:9 or SEQ ID NO:12, in which
an alkyl amino acid is substituted for an alkyl amino acid
20 in a Ztgf β -9 amino acid sequence, an aromatic amino acid
is substituted for an aromatic amino acid in an Ztgf β -9
amino acid sequence, a sulfur-containing amino acid is
substituted for a sulfur-containing amino acid in an
Ztgf β -9 amino acid sequence, a hydroxy-containing amino
25 acid is substituted for a hydroxy-containing amino acid in
a Ztgf β -9 amino acid sequence, an acidic amino acid is
substituted for an acidic amino acid in a Ztgf β -9 amino
acid sequence, a basic amino acid is substituted for a
basic amino acid in a Ztgf β -9 amino acid sequence, or a
30 dibasic monocarboxylic amino acid is substituted for a
dibasic monocarboxylic amino acid in an Ztgf β -9 amino acid
sequence.

Among the common amino acids, for example, a
35 "conservative amino acid substitution" is illustrated by a
substitution among amino acids within each of the
following groups: (1) glycine, alanine, valine, leucine,

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and isoleucine, (2) phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine. For example, variant Ztgf β -9
5 polypeptides that have an amino acid sequence that differs from either SEQ ID NOs:2, 3, 4, 5 or 12 can be obtained by substituting a threonine residue for Ser, by substituting a valine residue for Ile, by substituting an aspartate residue for Glu, or by substituting a valine residue for
10 Ile. Additional variants can be obtained by producing polypeptides having two or more of these amino acid substitutions.

15 Variants of either the human or the murine Ztgf β -9 can be devised by aligning the amino acid sequences of SEQ ID NO:3 and SEQ ID NO:12, and by noting any differences in the corresponding amino acid residues.

The BLOSUM62 table is an amino acid substitution
20 matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins (Henikoff and Henikoff, *Proc. Nat'l Acad. Sci. USA* 89:10915 (1992)). Accordingly, the BLOSUM62
25 substitution frequencies can be used to define conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. Although it is possible to design amino acid substitutions based solely upon chemical properties (as
30 discussed above), the language "conservative amino acid substitution" preferably refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0,
35 1, 2, or 3. According to this system, preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (e.g., 1, 2 or 3), while

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more preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 2 (e.g., 2 or 3).

5 Particular variants of human or murine Ztgfb β -9 are characterized by having at least 70%, at least 80%, at least 90%, at least 95% or 99% or greater sequence identity to the corresponding human (i.e., SEQ ID NOs: 2, 3, 4, 5 or 17) or murine (i.e., SEQ ID NOs: 9 or 12) amino
10 acid sequences, wherein the variation in amino acid sequence is due to one or more conservative amino acid substitutions.

 Conservative amino acid changes in a Ztgfb β -9
15 gene can be introduced by substituting nucleotides for the nucleotides recited in any one of SEQ ID NOs: 1 or 9. Such "conservative amino acid" variants can be obtained, for example, by oligonucleotide-directed mutagenesis, linker-scanning mutagenesis, mutagenesis using the polymerase
20 chain reaction, and the like (see Ausubel (1995) at pages 8-10 to 8-22; and McPherson (ed.), *Directed Mutagenesis: A Practical Approach* (IRL Press 1991)). The ability of such variants to promote anti-viral or anti-proliferative activity can be determined using a standard method, such
25 as the assay described herein. Alternatively, a variant Ztgfb β -9 polypeptide can be identified by the ability to specifically bind anti- Ztgfb β -9 antibodies.

 The proteins of the present invention can also
30 comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine,
35 hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-

methylethylproline, 3,3-dimethylethylproline, tert-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is typically carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., *J. Am. Chem. Soc.* 113:2722 (1991), Ellman et al., *Methods Enzymol.* 202:301 (1991), Chung et al., *Science* 259:806 (1993), and Chung et al., *Proc. Nat'l Acad. Sci. USA* 90:10145 (1993).

In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., *J. Biol. Chem.* 271:19991 (1996)). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide et al., *Biochem.* 33:7470 (1994). Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, *Protein Sci.* 2:395 (1993)).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for Ztgf β -9 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081 (1989), Bass et al., *Proc. Nat'l Acad. Sci. USA* 88:4498 (1991), Coombs and Corey, "Site-Directed Mutagenesis and Protein Engineering," in *Proteins: Analysis and Design*, Angeletti (ed.), pages 259-311 (Academic Press, Inc. 1998)). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity as disclosed below to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., *J. Biol. Chem.* 271:4699 (1996).

Table 2

25	<u>Conservative amino acid substitutions</u>	
	Basic:	arginine
		lysine
		histidine
	Acidic:	glutamic acid
30		aspartic acid
	Polar:	glutamine
		asparagine
	Hydrophobic:	leucine
		isoleucine
35		valine
	Aromatic:	phenylalanine
		tryptophan

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Small: tyrosine
glycine
alanine
serine
5 threonine
methionine

Essential amino acids in the polypeptides of the present invention can be identified according to
10 procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis [Cunningham and Wells, *Science* 244: 1081-1085 (1989); Bass et al., *Proc. Natl. Acad. Sci. USA* 88:4498-4502 (1991)]. In the latter technique, single alanine mutations are introduced
15 at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (e.g., ligand binding and signal transduction) to identify amino acid residues that are critical to the activity of the molecule. Sites of ligand-protein interaction can also be
20 determined by analysis of crystal structure as determined by such techniques as nuclear magnetic resonance, crystallography or photoaffinity labeling. See, for example, de Vos et al., *Science* 255:306-312 (1992); Smith et al., *J. Mol. Biol.* 224:899-904, 1992; Wlodaver et al.,
25 *FEBS Lett.* 309:59-64 (1992). The identities of essential amino acids can also be inferred from analysis of homologies with related proteins.

Multiple amino acid substitutions can be made
30 and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer, *Science* 241:53-57 (1988) or Bowie and Sauer, *Proc. Natl. Acad. Sci. USA* 86:2152-2156 (1989). Briefly, these authors disclose methods for simultaneously randomizing
35 two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of

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allowable substitutions at each position. Other methods that can be used include phage display, e.g., Lowman et al., *Biochem.* 30:10832-10837 (1991); Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis, Derbyshire et al., *Gene* 46:145 (1986); Ner et al., *DNA* 7:127 (1988).

Mutagenesis methods as disclosed above can be combined with high-throughput screening methods to detect activity of cloned, mutagenized proteins in host cells. Preferred assays in this regard include cell proliferation assays and biosensor-based ligand-binding assays, which are described below. Mutagenized DNA molecules that encode active proteins or portions thereof (e.g., ligand-binding fragments) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed above, one of ordinary skill in the art can prepare a variety of polypeptides that are substantially identical to SEQ ID NOS: 2, 3, 4, 5, 9, 12, 17 or 18 or allelic variants thereof and retain the properties of the wild-type protein. As expressed and claimed herein the language, "a polypeptide as defined by SEQ ID NOS: 2, 3, 4, 5, 9, 12, 17 or 18" includes all allelic variants and species orthologs of the polypeptide.

Another embodiment of the present invention provides for a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of the this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. A region of a protein to which an antibody can

bind is defined as an "antigenic epitope". See for instance, Geysen, H.M. et al., *Proc. Natl. Acad Sci. USA* 81:3998-4002 (1984).

5 As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in the art that relatively short synthetic peptides that mimic part of a protein sequence are
10 routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See Sutcliffe, J.G. et al. *Science* 219:660-666 (1983). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized
15 by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl termini. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at
20 inducing antibodies that bind to the mimicked protein; longer soluble peptides, especially those containing proline residues, usually are effective.

 Antigenic epitope-bearing peptides and
25 polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. Antigenic epitope-bearing peptides and polypeptides of the present invention contain a sequence of at least nine,
30 preferably between 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of the invention, containing from 30 to 50 amino acids, or any length up to
35 and including the entire amino acid sequence of a polypeptide of the invention, also are useful for inducing antibodies that react with the protein. Preferably, the

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amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (i.e., the sequence includes relatively hydrophilic residues and hydrophobic residues are preferably avoided); and sequences containing proline residues are particularly preferred. All of the polypeptides shown in the sequence listing contain antigenic epitopes to be used according to the present invention.

10

Polynucleotides, generally a cDNA sequence, of the present invention encode the above-described polypeptides. A cDNA sequence which encodes a polypeptide of the present invention is comprised of a series of codons, each amino acid residue of the polypeptide being encoded by a codon and each codon being comprised of three nucleotides. The amino acid residues are encoded by their respective codons as follows.

20

Alanine (Ala) is encoded by GCA, GCC, GCG or GCT;

25

Cysteine (Cys) is encoded by TGC or TGT;
Aspartic acid (Asp) is encoded by GAC or GAT;
Glutamic acid (Glu) is encoded by GAA or GAG;
Phenylalanine (Phe) is encoded by TTC or TTT;
Glycine (Gly) is encoded by GGA, GGC, GGG or GGT;

30

Histidine (His) is encoded by CAC or CAT;
Isoleucine (Ile) is encoded by ATA, ATC or ATT;
Lysine (Lys) is encoded by AAA, or AAG;
Leucine (Leu) is encoded by TTA, TTG, CTA, CTC, CTG or CTT;

35

Methionine (Met) is encoded by ATG;
Asparagine (Asn) is encoded by AAC or AAT;
Proline (Pro) is encoded by CCA, CCC, CCG or CCT;

Glutamine (Gln) is encoded by CAA or CAG;

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Arginine (Arg) is encoded by AGA, AGG, CGA, CGC, CGG or CGT;

Serine (Ser) is encoded by AGC, AGT, TCA, TCC, TCG or TCT;

5 Threonine (Thr) is encoded by ACA, ACC, ACG or ACT;

Valine (Val) is encoded by GTA, GTC, GTG or GTT;
Tryptophan (Trp) is encoded by TGG; and
Tyrosine (Tyr) is encoded by TAC or TAT.

10

It is to be recognized that according to the present invention, when a cDNA is claimed as described above, it is understood that what is claimed are both the sense strand, the anti-sense strand, and the DNA as
15 double-stranded having both the sense and anti-sense strand annealed together by their respective hydrogen bonds. Also claimed is the messenger RNA (mRNA) which encodes the polypeptides of the present invention, and which mRNA is encoded by the above-described cDNA. A
20 messenger RNA (mRNA) will encode a polypeptide using the same codons as those defined above, with the exception that each thymine nucleotide (T) is replaced by a uracil nucleotide (U).

25 The protein polypeptides of the present invention, including full-length proteins, protein fragments (e.g. receptor-binding fragments), and fusion polypeptides can be produced in genetically engineered host cells according to conventional techniques. Suitable
30 host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred.
35 Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., *Molecular Cloning*: A

Laboratory Manual, (2nd ed.) (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

In general, a DNA sequence encoding a Ztgf β -9 polypeptide is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a Ztgf β -9 polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of the protein, or may be derived from another secreted protein [e.g., the tissue plasminogen activator (t-PA)] leader sequence or synthesized *de novo*. The secretory signal sequence is joined to the Ztgf β -9 DNA sequence in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830).

35

Cultured mammalian cells are preferred hosts within the present invention. Methods for introducing

exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection, Wigler et al., *Cell* 14:725 (1978); Corsaro and Pearson, *Somatic Cell Genetics* 7:603 (1981); Graham and Van der Eb, *Virology* 52:456 (1973), electroporation, Neumann et al., *EMBO J.* 1:841-845 (1982), DEAE-dextran mediated transfection, Ausubel et al., eds., *Current Protocols in Molecular Biology* (John Wiley and Sons, Inc., NY, 1987), and liposome-mediated transfection (Hawley-Nelson et al., *Focus* 15:73 (1993); Ciccarone et al., *Focus* 15:80 (1993)). The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 [ATCC No. CRL 1573; Graham et al., *J. Gen. Virol.* 36:59-72 (1977)] and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

Other higher eukaryotic cells can also be used as hosts, including plant cells, insect cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., *J. Biosci. (Bangalore)* 11:47 (1987). Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222 and WIPO publication WO 94/06463.

Insect cells can be infected with recombinant baculovirus, commonly derived from *Autographa californica* nuclear polyhedrosis virus (AcNPV). DNA encoding the Ztgf β -9 polypeptide is inserted into the baculoviral genome in place of the AcNPV polyhedrin gene coding sequence by one of two methods. The first is the traditional method of homologous DNA recombination between wild-type AcNPV and a transfer vector containing the Ztgf β -9 cDNA flanked by AcNPV sequences. Suitable insect cells, e.g. SF9 cells, are infected with wild-type AcNPV and transfected with a transfer vector comprising a Ztgf β -9 polynucleotide operably linked to an AcNPV polyhedrin gene promoter, terminator, and flanking sequences. See, King, L.A. and Possee, R.D., *The Baculovirus Expression System: A Laboratory Guide*, (Chapman & Hall, London); O'Reilly, D.R. et al., *Baculovirus Expression Vectors: A Laboratory Manual* (Oxford University Press, New York, New York, 1994); and, Richardson, C. D., Ed., *Baculovirus Expression Protocols. Methods in Molecular Biology*, (Humana Press, Totowa, NJ 1995). Natural recombination within an insect cell will result in a recombinant baculovirus which contains Ztgf β -9 driven by the polyhedrin promoter. Recombinant viral stocks are made by methods commonly used in the art.

The second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow, V.A, et al., *J Virol* 67:4566 (1993). This system is sold in the Bac-to-Bac kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the Ztgf β -9 polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." The pFastBac1™ transfer vector utilizes the AcNPV polyhedrin promoter to drive the expression of the gene of interest, in this case Ztgf β -9.

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However, pFastBac1™ can be modified to a considerable degree. The polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as Pcor, p6.9 or MP promoter) which is

5 expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins. See, Hill-Perkins, M.S. and Possee, R.D., *J Gen Virol* 71:971 (1990); Bonning, B.C. et al., *J Gen Virol* 75:1551 (1994); and, Chazenbalk, G.D., and Rapoport, B., *J*

10 *Biol Chem* 270:1543 (1995). In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed which replace the native Ztgfβ-9 secretory signal sequences with secretory signal sequences derived

15 from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen, Carlsbad, CA), or baculovirus gp67 (PharMingen, San Diego, CA) can be used in constructs to replace the native Ztgfβ-9 secretory signal sequence.

20 In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed Ztgfβ-9 polypeptide, for example, a Glu-Glu epitope tag, Grussenmeyer, T. et al., *Proc Natl Acad Sci.* 82:7952 (1985). Using a technique

25 known in the art, a transfer vector containing Ztgfβ-9 is transformed into *E. coli*, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques,

30 and used to transfect *Spodoptera frugiperda* cells, e.g. Sf9 cells. Recombinant virus that expresses Ztgfβ-9 is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

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The recombinant virus is used to infect host cells, typically a cell line derived from the fall army worm, *Spodoptera frugiperda*. See, in general, Glick and Pasternak, *Molecular Biotechnology: Principles and Applications of Recombinant DNA*, ASM Press, Washington, D.C. (1994). Another suitable cell line is the High FiveTM cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent #5,300,435). Commercially available serum-free media are used to grow and maintain the cells. Suitable media are Sf900 IITM (Life Technologies) or ESF 921TM (Expression Systems) for the Sf9 cells; and ExcellTM 405 (JRH Biosciences, Lenexa, KS) or Express FiveTM (Life Technologies) for the *T. ni* cells. The cells are grown up from an inoculation density of approximately $2-5 \times 10^5$ cells to a density of $1-2 \times 10^6$ cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. The recombinant virus-infected cells typically produce the recombinant Ztgf β -9 polypeptide at 12-72 hours post-infection and secrete it with varying efficiency into the medium. The culture is usually harvested 48 hours post-infection. Centrifugation is used to separate the cells from the medium (supernatant). The supernatant containing the z*** polypeptide is filtered through micropore filters, usually 0.45 μ m pore size. Procedures used are generally described in available laboratory manuals (King, L. A. and Possee, R.D., *ibid.*; O'Reilly, D.R., et al., *ibid.*; Richardson, C. D., *ibid.*). Subsequent purification of the Ztgf β -9 polypeptide from the supernatant can be achieved using methods described herein.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been

inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as

5 "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the

10 gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high

15 levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, and puromycin acetyltransferase)

20 can also be used.

Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. Transformation of insect cells and production of

25 foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222; Bang et al., U.S. Patent No. 4,775,624; and WIPO publication WO 94/06463. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al.,

30 *J. Biosci. (Bangalore)* 11:47-58 (1987).

Fungal cells, including yeast cells, and particularly cells of the genus *Saccharomyces*, can also be used within the present invention, such as for producing

35 protein fragments or polypeptide fusions. Methods for transforming yeast cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for

example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075. Transformed
5 cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the *POT1* vector system disclosed by Kawasaki et al., U.S.
10 Patent No. 4,931,373, which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No.
15 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*,
20 *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., *J. Gen. Microbiol.* 132:3459-3465 (1986) and Cregg, U.S. Patent No. 4,882,279.
25 *Aspergillus* cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S.
30 Patent No. 4,486,533.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention.
35 Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art, see, e.g., Sambrook et al., *ibid.*). When expressing

a Ztgf β -9 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

Within one aspect of the present invention, a novel protein is produced by a cultured cell, and the cell or the protein is used to screen for a receptor or receptors for the protein, including the natural receptor,

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as well as interacting proteins such as dimerization partners, agonists and antagonists of the natural ligand.

PROTEIN ISOLATION:

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Expressed recombinant polypeptides (or chimeric polypeptides) can be purified using fractionation and/or conventional purification methods and media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable anion exchange media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred, with DEAE Fast-Flow Sepharose (Pharmacia, Piscataway, NJ) being particularly preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from

commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988).

The polypeptides of the present invention can be isolated by exploitation of their properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins. Briefly, a gel is first charged with divalent metal ions to form a chelate [E. Sulkowski, *Trends in Biochem.* 3:1-7 (1985)]. Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography [Methods in Enzymol., Vol. 182:529-39, "Guide to Protein Purification", M. Deutscher, (ed.), (Acad. Press, San Diego, 1990). Alternatively, a fusion of the polypeptide of interest and an affinity tag (e.g., polyhistidine, maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification. Furthermore, to facilitate purification of the secreted polypeptide, an amino or carboxyl-terminal extension, such as a poly-histidine tag, substance P, FLAG[®] peptide [Hopp et al., *Bio/Technology* 6:1204-1210 (1988); available from Eastman Kodak Co., New Haven, CT), a Glu-Glu affinity tag [Grussenmeyer et al., *Proc. Natl. Acad. Sci. USA* 82:7952-4 (1985)], or another polypeptide or protein for which an antibody or other specific binding agent is available, can be fused to Ztgf β to aid in purification.

Mice engineered to express the *Ztgfβ-9* gene, referred to as "transgenic mice" and mice that exhibit a complete absence of *Ztgfβ-9* gene function, referred to as "knockout mice", may also be generated (Snouwaert et al., *Science*, 257:1083 (1992); Lowell, et al., *Nature*, 366:740-742 (1993); Capecchi, M.R., *Science*, 244:1288-1292, (1989); Palmiter, R.D. et al., *Annu. Rev. Genet.*, 20:465-499, (1986). For example, transgenic mice that over-express *Ztgfβ-9* either ubiquitously or under a tissue-specific or tissue-restricted promoter can be used to ask whether overexpression causes a phenotype. For example, overexpression of a wild-type *Ztgfβ-9* polypeptide, polypeptide fragment or a mutant thereof may alter normal cellular processes resulting in a phenotype that identifies a tissue in which *Ztgfβ-9* expression is functionally relevant and may indicate a therapeutic target for the *Ztgfβ-9* protein, gene, its agonists or antagonists. Moreover, such over-expression may result in a phenotype that shows similarity with human diseases. Similarly, knockout *Ztgfβ-9* mice can be used to determine where *Ztgfβ-9* is absolutely required *in vivo*. The phenotype of knockout mice is predictive of the *in vivo* effects of that of a *Ztgfβ-9* antagonist. The human *Ztgfβ-9* cDNA can be used to isolate murine *Ztgfβ-9* mRNA, cDNA and genomic DNA, which are subsequently used to generate knockout or transgenic mice. These mice may be employed to study the *Ztgfβ-9* gene and the protein encoded thereby in an *in vivo* system, and can be used as *in vivo* models for corresponding human diseases. Moreover, transgenic mouse expression of *Ztgfβ-9* antisense polynucleotides or ribozymes directed against *Ztgfβ-9* or single chain antibodies to *Ztgfβ-9* can be used to further elucidate the biology of *Ztgfβ-9*.

Northern blot analysis of the expression of Ztgf β -9 reveals that Ztgf β -9 is highly expressed in the brain and spinal cord. Therefore, Ztgf β -9 may play a role in the maintenance of spinal cord involving either glial cells or neurons. This indicates that Ztgf β -9 can be used to treat a variety of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Huntington's disease, Parkinson's disease and peripheral neuropathies, or demyelinating diseases including multiple sclerosis. The tissue specificity of Ztgf β -9 expression suggests that Ztgf β -9 may be a growth and/or maintenance factor in the spinal cord and brain which can be used to treat spinal cord, brain or peripheral nervous system injuries. Ztgf β -9 can also be administered to someone to treat a viral infection.

The present invention also provides reagents with significant therapeutic value. The Ztgf β -9 polypeptide (naturally occurring or recombinant), fragments thereof, antibodies and anti-idiotypic antibodies thereto, along with compounds identified as having binding affinity to the Ztgf β -9 polypeptide, should be useful in the treatment of conditions associated with abnormal physiology or development, including abnormal proliferation, e.g., cancerous conditions, or degenerative conditions. For example, a disease or disorder associated with abnormal expression or abnormal signaling by a Ztgf β -9 polypeptide should be a likely target for an agonist or antagonist of the Ztgf β -9 polypeptide. In particular, Ztgf β -9 can be used to treat inflammation. Inflammation is a result of an immune response to an infection or as an autoimmune response to a self-antigen.

Antibodies to the Ztgf β -9 polypeptide can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in

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pharmaceutically acceptable carriers or diluents along with physiologically innocuous stabilizers and excipients. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies, binding fragments thereof or single-chain antibodies of the antibodies including forms which are not complement binding.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medications administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in vivo* administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Methods for administration include oral, intravenous, peritoneal, intramuscular, or transdermal administration. Pharmaceutically acceptable carriers will include water, saline or buffers to name just a few. Dosage ranges would ordinarily be expected from 1 μ g to 1000 μ g per kilogram of body weight per day. However, the doses may be higher or lower as can be determined by a medical doctor with ordinary skill in the art. For a complete discussion of drug formulations and dosage ranges see *Remington's Pharmaceutical Sciences*, 17th Ed., (Mack Publishing Co., Easton, Penn., 1990), and *Goodman and Gilman's: The Pharmacological Bases of Therapeutics*, 9th Ed. (Pergamon Press 1996).

Nucleic Acid-based Therapeutic Treatment

If a mammal has a mutated or lacks a *Ztgfβ-9* gene, the *Ztgfβ-9* gene can be introduced into the cells of the mammal. In one embodiment, a gene encoding a *Ztgfβ-9* polypeptide is introduced *in vivo* in a viral vector. Such vectors include an attenuated or defective DNA virus, such as but not limited to herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), SV40 and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. A defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Examples of particular vectors include, but are not limited to, a defective herpes virus 1 (HSV1) vector [Kaplitt et al., *Molec. Cell. Neurosci.*, 2 :320-330 (1991)], an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., *J. Clin. Invest.*, 90 :626-630 (1992), and a defective adeno-associated virus vector [Samulski et al., *J. Virol.*, 61:3096-3101 (1987); Samulski et al. *J. Virol.*, 63:3822-3828 (1989)].

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In another embodiment, the gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent No. 5,399,346; Mann et al., *Cell*, 33:153 (1983); Temin et al., U.S. Patent No. 4,650,764; Temin et al., U.S. Patent No. 4,980,289; Markowitz et al., *J. Virol.*, 62:1120 (1988); Temin et al., U.S. Patent No. 5,124,263; International Patent Publication No. WO 95/07358, published March 16, 1995 by Dougherty et al.; and Blood, 82:845 (1993).

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Alternatively, the vector can be introduced by lipofection *in vivo* using liposomes. Synthetic cationic

lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker [Felgner et al., *Proc. Natl. Acad. Sci. USA*, 84:7413-7417 (1987); see Mackey et al., *Proc. Natl. Acad. Sci. USA*, 85:8027-8031 (1988)]. The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. It is clear that directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides, e.g., hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

It is possible to remove the cells from the body and introduce the vector as a naked DNA plasmid or by means of a viral vector and then re-implant the transformed cells into the body. Naked DNA vector for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter [see, e.g., Wu et al., *J. Biol. Chem.*, 267:963-967 (1992); Wu et al., *J. Biol. Chem.*, 263:14621-14624 (1988)]. Techniques such as viral vector-mediated gene delivery of Ztgf β -9 can be used to treat human diseases such as cancer, immune & autoimmune diseases, and diseases of the central and peripheral nervous system.

Ztgf β -9 polypeptides can also be used to prepare antibodies that specifically bind to Ztgf β -9 polypeptides. These antibodies can then be used to manufacture anti-idiotypic antibodies. As used herein, the term

"antibodies" includes polyclonal antibodies, monoclonal antibodies, antigen-binding fragments thereof such as $F(ab')_2$ and Fab fragments, and the like, including genetically engineered antibodies. Antibodies are defined to be specifically binding if they bind to a Ztgf β -9 polypeptide with a K_a of greater than or equal to $10^7/M$ and they do not substantially bind to a polypeptide of the prior art. The affinity of a monoclonal antibody can be readily determined by one of ordinary skill in the art, for example, by using Scatchard analysis.

Methods for preparing polyclonal and monoclonal antibodies are well known in the art (see for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, (Second Edition) (Cold Spring Harbor, NY, 1989); and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications* (CRC Press, Inc., Boca Raton, FL, 1982). Polyclonal antibodies can be generated by inoculating a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, hamsters, guinea pigs and rats with a Ztgf β -9 polypeptide or a fragment thereof. The immunogenicity of a Ztgf β -9 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of Ztgf β -9 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization. A variety of assays known to those skilled in the art can be utilized to detect antibodies which specifically bind to Ztgf β -9 polypeptides. Exemplary assays are described in detail in

Antibodies: A Laboratory Manual, Harlow and Lane (Eds.), (Cold Spring Harbor Laboratory Press, 1988).

Representative examples of such assays include: concurrent immunoelectrophoresis, radio-immunoassays, radio-
5 immunoprecipitations, enzyme-linked immunosorbent assays (ELISA), dot blot assays, inhibition or competition assays, and sandwich assays.

As used herein, the term "antibodies" includes
10 polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as F(ab')₂ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain
15 antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains
20 (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper
25 binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced. Human antibodies can be generated in mice engineered to contain the human immunoglobulin loci,
30 Vaughan, et al. *Nat. Biotech.*, 16:535-539 (1998).

Alternative techniques for generating or selecting antibodies useful herein include in vitro exposure of lymphocytes to Ztgf β -9 protein or peptide, and
35 selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized

or labeled Ztgf β -9 protein or peptide). Genes encoding polypeptides having potential Ztgf β -9 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on
5 bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides which interact with a known
10 target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner et al., US
15 Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and Ladner et al., US Patent NO. 5,571,698) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech
20 (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the Ztgf β -9 sequences disclosed herein to identify proteins which bind
25 to Ztgf β -9. These "binding proteins" which interact with Ztgf β -9 polypeptides can be used for tagging cells; for isolating homolog polypeptides by affinity purification; they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding
30 proteins can also be used in analytical methods such as for screening expression libraries and neutralizing activity. The binding proteins can also be used for diagnostic assays for determining circulating levels of polypeptides; for detecting or quantitating soluble
35 polypeptides as marker of underlying pathology or disease. These binding proteins can also act as Ztgf β -9

"antagonists" to block Ztgf β -9 binding and signal transduction *in vitro* and *in vivo*.

Antibodies can also be generated by gene therapy. The animal is administered the DNA or RNA which encodes Ztgf β -9 or an immunogenic fragment thereof so that cells of the animals are transfected with the nucleic acid and express the protein which in turn elicits an immunogenic response. Antibodies which then are produced by the animal are isolated in the form of polyclonal or monoclonal antibodies. Antibodies to Ztgf β -9 may be used for tagging cells that express the protein, for affinity purification, within diagnostic assays for determining circulating levels of soluble protein polypeptides, and as antagonists to block ligand binding and signal transduction *in vitro* and *in vivo*.

Radiation hybrid mapping is a somatic cell genetic technique developed for constructing high-resolution, contiguous maps of mammalian chromosomes [Cox et al., *Science* 250:245-250 (1990)]. Partial or full knowledge of a gene's sequence allows the designing of PCR primers suitable for use with chromosomal radiation hybrid mapping panels. Commercially available radiation hybrid mapping panels which cover the entire human genome, such as the Stanford G3 RH Panel and the GeneBridge 4 RH Panel (Research Genetics, Inc., Huntsville, AL), are available. These panels enable rapid, PCR based, chromosomal localizations and ordering of genes, sequence-tagged sites (STSS), and other nonpolymorphic- and polymorphic markers within a region of interest. This includes establishing directly proportional physical distances between newly discovered genes of interest and previously mapped markers. The precise knowledge of a gene's position can be useful in a number of ways including: 1) determining if a sequence is part of an existing contig and obtaining

additional surrounding genetic sequences in various forms such as genomic YAC-, BAC- or phage genomic clones or cDNA clones, 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region, and 3) for cross-referencing model organisms such as mouse which may be beneficial in helping to determine what function a particular gene might have.

The present invention also provides reagents which will find use in diagnostic applications. For example, the *Ztgfb-9* gene has been mapped on chromosome 13q11.2-q11. A *Ztgfb-9* nucleic acid probe could be used to check for abnormalities on chromosome 13. For example, a probe comprising *Ztgfb-9* DNA or RNA or a subsequence thereof can be used to determine if the *Ztgfb-9* gene is present on human chromosome 13q11.2-q11 or if a mutation has occurred. Detectable chromosomal aberrations at the *Ztgfb-9* gene locus include but are not limited to aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art. Human *Ztgfb-9* maps at the 13q11.2-q11 region. Mouse *Ztgfb-9* maps to mouse chromosome 14 framework markers d14mit64 and dmit82 located at 22.0 and 19.5 centimorgans, respectively. The 19.5 cm region appears to be syntenic with the human locus containing the gap junction genes *gja3* and *gjb2*. See Mignon, C. et al., *Cytogenet. Cell Genet.* 72: 185-186 (1996).

The invention is further illustrated by the following non-limiting examples.

Example 1.Cloning of *Ztgf β -9*

5 Human *Ztgf β -9* was isolated from an arrayed pituitary gland cDNA plasmid library by PCR screening using SEQ ID NOs: 6 and 7. Thermocycler conditions were as follows: one cycle at 94°C for 3 minutes, 35 cycles at 94°C for 30 seconds, 62°C for 20 seconds, 72°C for 30 seconds, one
10 cycle at 72°C for 5 minutes, followed by 4° C hold. The reactions were gel electrophoresed to identify positive pools and, in this way the library was deconvoluted to a pool of positive clones. These were electroporated into *E. coli* DH10B cells and plated for colony hybridization. The
15 colonies were transferred to Hybond N filters (Amersham) and probed for positive colonies. Positive clones were sequenced for full length *Ztgf β -9*.

Sequence analysis and conceptual translation of the
20 human *Ztgf β -9* cDNA (SEQ ID NOs: 1 and 16) predicts a protein product that is 202 amino acid residues in length. This protein is homologous to two members of the IL-17 family, Zcyto7, International Application No. PCT/US98/08212, and IL-17. *Ztgf β -9* shares 27.8% amino acid
25 identity with Zcyto7 and 20.6% identity with IL-17 as determined by the Clustal Method using Lasergene MegAlign software. See Higgins and Sharp, *CABIOS* 5:151 (1989). In particular, *Ztgf β -9* shares four conserved cysteines (amino acid residues 114, 119, 167, 169 in SEQ ID NO:2) with
30 Zcyto7 and IL-17. These cysteines are predicted to be involved in forming a cysteine-knot-like protein fold that is related to the found in TGF- β proteins.

Example 2

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Northern Analysis *Ztgf β -9*

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Analysis of tissue distribution was performed by the northern blotting technique using 2 adult and 1 fetal human brain blots, Human Multiple Tissue and Master Dot Blots from Clontech (Palo Alto, Ca). A probe was
5 obtained by PCR using SEQ ID NOs:6 and 7. in a cDNA pool. Thermocycler conditions were as follows: one cycle at 94°C for 3 minutes, 35 cycles at 94°C for 10 seconds, 66°C for 20 seconds, 72°C for 30 seconds, one cycle at 72°C for 5 minutes, followed by 4°C hold. The reaction mixture was
10 electrophoresed on a preparative agarose gel and a 162 bp fragment was gel purified using commercially available gel purification reagents and protocol (QIAEX II Gel Extraction Kit; Qiagen, Inc., Santa Clarita, Ca). The purified DNA was radioactively labeled with ³²P using a
15 commercially available kit (Rediprime DNA labeling system; Amersham Corp., Arlington Heights, IL). The probe was purified using a NUCTRAP push column (Stratagene Cloning Systems, La Jolla, CA). EXPRESSHYB (Clontech, Palo Alto, CA) solution was used for prehybridization and
20 hybridization. The hybridization solution consisted of 8 mls EXPRESSHYB, 80µl Sheared Salmon Sperm DNA (10mg/ml, 5 Prime-3 Prime, Boulder, CO), 48 µl Human Cot-1 DNA (1mg/ml, GibcoBRL) and 18µl of radiolabeled probe. Hybridization took place overnight at 50°C And the blots
25 were then washed in 2X SSC, 0.1% SDS at RT, then 2X SSC, 0.1% SDS at 60°C, followed by 0.1X SSC, 0.1% SDS wash at 60°C. The blots were exposed overnight and developed. A major transcript signal of was observed on MTN blots in brain and spinal cord.

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Master Dot blot signals were strong in all brain tissues (adult and fetal), spinal cord, heart, skeletal muscle, stomach, pancreas, adrenal gland, salivary gland, liver, small intestine, bone marrow, thymus, spleen, lymph
35 node, heart, thyroid, trachea, testis, ovary and placenta.

Example 3

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Cloning of Murine *Ztgfb-9*

Full length sequence was obtained from a clone
5 isolated from an arrayed mouse testis cDNA/plasmid
library. The library was screened by PCR using
oligonucleotides SEQ NO:10 and SEQ ID NO:11. The library
was deconvoluted down to a positive pool of 250 clones.
E.coli DH10B cells (Gibco BRL) were transformed with this
10 pool by electroporation. The transformed culture was
titered and arrayed out to 96 wells at ~20 cells/well. The
cells were grown up in LBamp overnight at 37°C. An aliquot
of the cells were pelleted and PCR was used to identify a
positive pool. The remaining cells from a positive pool
15 were plated and colonies screened by PCR to identify a
positive clone. The clone was sequenced and contained the
putative full length sequence of murine *Ztgfb-9*. The
sequence of murine *Ztgfbeta-9* is defined by SEQ ID NOS: 8
and 9.

20

Example 4Northern Analysis of Mouse *Ztgfb-9*

25 Northern analysis was also carried out on Mouse MTN
and Master Dot blots (Clontech) and a Mouse Embryo blot.
A full-length murine *Ztgfb-9* cDNA clone (see cloning
section) was restriction digested with *ApaI* and *EcoRI*
following standard protocols. The reaction was gel
30 electrophoresed and the ~686bp fragment was gel purified
using the Qiaex II Gel Purification Kit (Qiagen, Valencia,
CA). The cDNA was P32-labeled using the Rediprime II
Labeling Kit (Amersham) and column purified using reagents
and protocols described earlier. Hybridization, washing,
35 and detection were carried out under conditions as
described in Example 2. A band was observed in heart,
brain, lung, liver, skeletal muscle, kidney and testis.

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The Master Dot blot had strong signals in thyroid, with fainter signals in most other tissues. Hybridization to the Mouse Embryo blot indicated that Ztgfb β -9 was expressed at all stages examined (embryonic days 7, 11, 15, and 17).

5

By quantitative RT-PCR, murine Ztgfb β -9 was found to be highly expressed in the HCL hypothalamic cell line, and at lower levels in the GT1-1 and GT1-7 hypothalamic cell lines and the undifferentiated P19 teratocarcinoma cell line. Using quantitative RT-PCR, murine Ztgfb β -9 was detected in neurons of the hippocampal cerebellar and olfactory cortex, Purkinje cells and other neuronal populations were heavily labeled in brain sections. The endothelium of the choroid plexus was also heavily positive. In the spinal cord, labeling was confined to the gray matter and appeared to be uniformly found in dorsal and ventral horn neurons representing sensory and motor neurons. Strong expression was also observed in the dorsal root ganglia.

20

Example 5

Antibody Production

5 Polypeptides SEQ ID NOs: 13, 14 and 15 were
synthesized and were injected into rabbits and polyclonal
anti-sera was subsequently affinity purified by column
chromatography using the cognate immunogen. Also fusion
proteins between full-length human and mouse Ztgf- β fused
10 to the C-terminus of the maltose-binding protein were
expressed in *E. coli* and purified by affinity
chromatography over an amylose resin. Purified proteins
were injected into rabbits and polyclonal anti-sera was
subsequently affinity purified by column chromatography
15 using the cognate immunogen.

Example 6

Immunocytochemistry

20 Affinity purified polyclonal antibodies to human
Ztgf β -9 produced according to the procedure of Example 5
were validated on normal COS cells and COS cells
transfected with a Ztgf β -9 mammalian cell expression
25 construct. Immunocytochemistry performed with anti-Ztgf β -9
antibodies demonstrated Ztgf β -9 expression in monkey brain
and spinal cord. The immunocytochemistry staining was
intracytoplasmic and observed in many large neurons and
Purkinje cells. Scattered epithelial cells in the human
30 duodenum also showed positive staining.

Example 7

Mammalian cell protein production

35 Human Ztgf β -9 protein, both with and without a
C-terminal a Glu-Glu affinity tag [Grussenmeyer et al.,

Proc. Natl. Acad. Sci. USA 82:7952-4 (1985)], was expressed in BHK cells using an expression vector in which Ztgf β -9 expression is driven by the CMV immediate early promoter, a consensus intron from the variable region of mouse immunoglobulin heavy chain locus, multiple restriction sites for insertion of coding sequences, a stop codon and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator and Kozac sequences at the 5' end of the open reading frame.

Following selection for stable cell transfectants, media isolated from pools was analyzed by western blot under reducing and non-reducing conditions using a Ztgf β -9 antibody or an anti-EE epitope tag antibody. Human Ztgf β -9 was found to migrate under reducing conditions at 29 kDa. Since the predicted molecular weight of the fully processed form of the protein is 20.31 kDa, this suggests that the protein is glycosylated at one or both of two potential glycosylation sites. Under non-reducing conditions, Ztgf β -9 protein migrated as at 49 kDa species. These results indicate that human Ztgf β -9 is capable of forming a disulfide cross-linked homodimer. However, co-expression of C-terminally EE tagged human Ztgf β -9 and untagged human Zcyto7, followed by affinity purification using an anti-EE tag antibody resulted in co-purification of both proteins, suggesting that in addition to Ztgf β -9 homodimers, Ztgf β -9 and Zcyto7 can also dimerize. Interaction of Ztgf β -9 and Zcyto7 did not appear to be due to interchain disulfide-bonding between the proteins. C-terminal EE tagged human Ztgf β -9 expressed in BHK cells was also anti-EE affinity purified and its N-terminal sequence determined. The signal cleavage site was found to occur proceeding amino acid A23.

Example 8

5

Transgenic mice

The open reading frame encoding full-length murine Ztgf β -9 was amplified by PCR so as to introduce an
10 optimized initiation codon and introduced into a transgenic vector in which expression of Ztgf β -9 was regulated by the metallothionein I promoter. The transgene insert was separated from plasmid backbone by NotI digestion and agarose gel purification, and
15 fertilized ova from matings of B6C3F1Tac mice were microinjected and implanted into pseudopregnant females. Founders were identified by PCR on genomic tail DNA (DNAeasy 96 kit; Qiagen). Transgenic lines were initiated by breeding founders with C57BL/6Tac mice. Animal
20 protocols used in this study were approved by the ZymoGenetics Institutional Animal Care and Use Committee. From 49 progeny born only 8% were found to be transgenic (compared to an average of 20% observed for a variety of other cDNAs driven by the same promoter), suggesting that
25 high expression of murine Ztgf β -9 may be embryonic lethal. Consistent with this, of the four founders identified, all expressed only low levels of Ztgf β -9 mRNA in the liver. A fifth founder died at birth and interestingly, this animal expressed very high levels of
30 Ztgf β -9 mRNA in the liver (8500 copies/cell). Histopathological analysis of this animal identified severe apoptosis of the thymus and complete devaculization of brown fat. The expressing males were bred with wild-type females. One founder was capable of germline
35 transmission, however all transgenic progeny of this founder either died at birth or were runted and died soon after weaning. Analysis of these animals identified a

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variety of phenotypes, including severe thymic apoptosis, devaculization of brown fat, liver hepatitis, and low lymphocyte peripheral blood cell numbers. These results indicate that Ztgf β -9, its agonists and antagonists, and antibodies to Ztgf β -9 will be useful in regulating immune cells, adipogenesis, and liver cells.

Example 9

10 Chromosomal position

Human Ztgf β -9 was mapped to chromosome 13q11.2 on two radiation hybrid panels. The mouse Ztgfb9 gene links to murine chromosome 14 framework markers d14mit64 and dmit82 located at 22.0 and 19.5 centimorgans, respectively.

Example 10

Inhibition of Adenovirus Growth by Ztgf β -9

20

The human and murine Ztgf β -9 coding region were cloned into the adenovirus shuttle vector and recombined to generated the recombinant adenoviral genome. Transfection of Ztgf β -9 adenoviral genomes into 293A cells resulted in very small viral plaques (1 or 2 plaques per transfection which is low). These plaques did not expand in size. Normally plaques expand greatly in size over a period of 1-2 days as the virus replicates. The small plaques were harvested and we tried to expand the virus by infecting 293A cells. Infected monolayers again exhibited very low numbers of plaques and the plaques were small in size. These plaques did not expand in size over time. After 2-3 rounds of attempting to amplify the virus, eventually we did obtain a rapidly growing virus population. The virus that results from this amplification still contains the Ztgf β -9 sequences. However, infection of cell by these viruses did not result

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in protein production. The initial behavior of both the mouse and human *Ztgf β -9*-containing viruses is unlike we have seen with any other cDNA. Clearly, virus replication was inhibited.

5

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1

SEQUENCE LISTING

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 1201 Eastlake Avenue East
 Seattle, Washington 98102
 United States of America

<120> Mammalian Transforming Growth Factor
 Beta - 9

<130> 98-54PC

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Trp Ala Tyr Arg Ile Ser Tyr Asp Pro Ala Arg Tyr Pro Arg Tyr Leu			
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Lys Leu Leu Leu Gly Pro Asn Asp Ala Pro Ala Gly Pro			
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3

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          85           90           95
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Tyr Cys Leu Cys Arg Gly Cys Leu Thr Gly Leu Phe Gly Glu Glu Asp
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145          150          155          160
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Ala Asp Ser Ile Asn Ser Ser Ile Asp Lys Gln Gly Ala Lys Leu Leu
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4

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Tyr	Arg	Ile	Ser	Tyr	Asp	Pro	Ala	Arg	Tyr	Pro	Arg	Tyr	Leu	Pro	Glu
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		35					40					45			
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5

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Tyr Cys Leu Cys Arg Gly Cys Leu Thr Gly Leu Phe Gly Glu Glu Asp
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      35              40              45
Ala Arg Asn Ala Ser Cys Pro Ala Gly Gly Arg Pro Ala Asp Arg Arg
      50              55              60
Phe Arg Pro Pro Thr Asn Leu Arg Ser Val Ser Pro Trp Ala Tyr Arg
65              70              75              80
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Cys Leu Cys Arg Gly Cys Leu Thr Gly Leu Phe Gly Glu Glu Asp Val
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6

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7

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ccc acc aac ctg cgc agc gtg tgc ccc tgg gcg tac agg att tcc tac			399
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8

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Asp	Lys	Gln	Gly	Ala	Lys	Leu	Leu	Leu	Gly	Pro	Asn	Asp	Ala	Pro	Ala
			20					25					30		
Gly	Pro														

<210> 16

<211> 2361

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (572)...(1202)

<400> 16

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aaaatagaga	tttaaaaaaa	cctctaattt	cctcgaagta	cagaatctca	agaggtagct	180
ctaaggagaa	tcctctggg	tttgagcgca	ttcctcttcc	agggggccta	ttcttgact	240

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gctttcctta atagagaaat ctctctgagc caaaatcggc ctccccaat tccatcctgt	300
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cgggcccgca cccgcgcccgc gcagggccag ccctctctc ctctgctg gcgcagcaca	420
ggccctgagc gcgcgacccc aggccctggg cgccccgcgc catgctcgcg gctggaagcc	480
ccagtttgcg tggcccttcg gggtattccg ctcaagagcc gccgcgtcgc cccatctcgc	540
cgcaatctg aaagcgcttt cgggggagaa g atg ttg ggg gca ctg gtc tgg	592
Met Leu Gly Ala Leu Val Trp	
1 5	
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Met Leu Val Ala Gly Phe Leu Leu Ala Leu Pro Pro Ser Trp Ala Ala	
10 15 20	
ggc gcc ccg agg gcg ggc agg cgc ccc gcg cgg ccg cgg ggc tgc gcg	688
Gly Ala Pro Arg Ala Gly Arg Arg Pro Ala Arg Pro Arg Gly Cys Ala	
25 30 35	
gac cgg ccg gag gag cta ctg gag cag ctg tac ggg cgc ctg gcg gcc	736
Asp Arg Pro Glu Glu Leu Leu Glu Gln Leu Tyr Gly Arg Leu Ala Ala	
40 45 50 55	
ggc gtg ctc agt gcc ttc cac cac acg ctg cag ctg ggg ccg cgt gag	784
Gly Val Leu Ser Ala Phe His His Thr Leu Gln Leu Gly Pro Arg Glu	
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Gln Ala Arg Asn Ala Ser Cys Pro Ala Gly Gly Arg Pro Ala Asp Arg	
75 80 85	
cgc ttc cgg ccg ccc acc aac ctg cgc agc gtg tgc ccc tgg gcc tac	880
Arg Phe Arg Pro Pro Thr Asn Leu Arg Ser Val Ser Pro Trp Ala Tyr	
90 95 100	
aga atc tcc tac gac ccg gcg agg tac ccc agg tac ctg cct gaa gcc	928
Arg Ile Ser Tyr Asp Pro Ala Arg Tyr Pro Arg Tyr Leu Pro Glu Ala	
105 110 115	
tac tgc ctg tgc cgg ggc tgc ctg acc ggg ctg ttc ggc gag gag gac	976
Tyr Cys Leu Cys Arg Gly Cys Leu Thr Gly Leu Phe Gly Glu Glu Asp	
120 125 130 135	
gtg cgc ttc cgc agc gcc cct gtc tac atg ccc acc gtc gtc ctg cgc	1024
Val Arg Phe Arg Ser Ala Pro Val Tyr Met Pro Thr Val Val Leu Arg	
140 145 150	

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cgc acc ccc gcc tgc gcc ggc ggc cgt tcc gtc tac acc gag gcc tac 1072
 Arg Thr Pro Ala Cys Ala Gly Gly Arg Ser Val Tyr Thr Glu Ala Tyr
 155 160 165

gtc acc atc ccc gtg ggc tgc acc tgc gtc ccc gag ccg gag aag gac 1120
 Val Thr Ile Pro Val Gly Cys Thr Cys Val Pro Glu Pro Glu Lys Asp
 170 175 180

gca gac agc atc aac tcc agc atc gac aaa cag ggc gcc aag ctc ctg 1168
 Ala Asp Ser Ile Asn Ser Ser Ile Asp Lys Gln Gly Ala Lys Leu Leu
 185 190 195

ctg ggc ccc aac gac gcg ccc gct ggc ccc tga g gccggctctg 1212
 Leu Gly Pro Asn Asp Ala Pro Ala Gly Pro *
 200 205

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<210> 17

<211> 209

<212> PRT

<213> Homo sapiens

<400> 17

Met Leu Gly Ala Leu Val Trp Met Leu Val Ala Gly Phe Leu Leu Ala
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 20 25 30
 Ala Arg Pro Arg Gly Cys Ala Asp Arg Pro Glu Glu Leu Leu Glu Gln
 35 40 45
 Leu Tyr Gly Arg Leu Ala Ala Gly Val Leu Ser Ala Phe His His Thr
 50 55 60
 Leu Gln Leu Gly Pro Arg Glu Gln Ala Arg Asn Ala Ser Cys Pro Ala
 65 70 75 80
 Gly Gly Arg Pro Ala Asp Arg Arg Phe Arg Pro Pro Thr Asn Leu Arg
 85 90 95
 Ser Val Ser Pro Trp Ala Tyr Arg Ile Ser Tyr Asp Pro Ala Arg Tyr
 100 105 110
 Pro Arg Tyr Leu Pro Glu Ala Tyr Cys Leu Cys Arg Gly Cys Leu Thr
 115 120 125
 Gly Leu Phe Gly Glu Glu Asp Val Arg Phe Arg Ser Ala Pro Val Tyr
 130 135 140
 Met Pro Thr Val Val Leu Arg Arg Thr Pro Ala Cys Ala Gly Gly Arg
 145 150 155 160
 Ser Val Tyr Thr Glu Ala Tyr Val Thr Ile Pro Val Gly Cys Thr Cys
 165 170 175
 Val Pro Glu Pro Glu Lys Asp Ala Asp Ser Ile Asn Ser Ser Ile Asp
 180 185 190
 Lys Gln Gly Ala Lys Leu Leu Leu Gly Pro Asn Asp Ala Pro Ala Gly
 195 200 205
 Pro

<210> 18
 <211> 187
 <212> PRT
 <213> Homo sapiens

<400> 18
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 Ala Asp Arg Pro Glu Glu Leu Leu Glu Gln Leu Tyr Gly Arg Leu Ala
 20 25 30
 Ala Gly Val Leu Ser Ala Phe His His Thr Leu Gln Leu Gly Pro Arg
 35 40 45
 Glu Gln Ala Arg Asn Ala Ser Cys Pro Ala Gly Gly Arg Pro Ala Asp
 50 55 60
 Arg Arg Phe Arg Pro Pro Thr Asn Leu Arg Ser Val Ser Pro Trp Ala
 65 70 75 80
 Tyr Arg Ile Ser Tyr Asp Pro Ala Arg Tyr Pro Arg Tyr Leu Pro Glu
 85 90 95

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Ala Tyr Cys Leu Cys Arg Gly Cys Leu Thr Gly Leu Phe Gly Glu Glu
 100 105 110
 Asp Val Arg Phe Arg Ser Ala Pro Val Tyr Met Pro Thr Val Val Leu
 115 120 125
 Arg Arg Thr Pro Ala Cys Ala Gly Gly Arg Ser Val Tyr Thr Glu Ala
 130 135 140
 Tyr Val Thr Ile Pro Val Gly Cys Thr Cys Val Pro Glu Pro Glu Lys
 145 150 155 160
 Asp Ala Asp Ser Ile Asn Ser Ser Ile Asp Lys Gln Gly Ala Lys Leu
 165 170 175
 Leu Leu Gly Pro Asn Asp Ala Pro Ala Gly Pro
 180 185

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 <211> 54
 <212> PRT
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<400> 19
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 35 40 45
 Arg Tyr Leu Pro Glu Ala
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 <212> PRT
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 <212> PRT
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<400> 21
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 1 5 10 15

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Tyr Val Thr Ile Pro Val Gly Cys Thr Cys Val Pro Glu Pro Glu Lys
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Asp Ala Asp Ser Ile Asn Ser Ser Ile Asp Lys Gln Gly Ala Lys Leu
35 40 45
Leu Leu Gly Pro Asn Asp Ala Pro Ala
50 55

<210> 22

<211> 14

<212> PRT

<213> Homo sapiens

<400> 22

Ser Tyr Asp Pro Ala Arg Tyr Pro Arg Tyr Leu Pro Glu Ala
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